Comparing Phylogenetic Codivergence between Polyomaviruses and Their Hosts

Marcos Pérez-Losada,[1]* Ryan G. Christensen,[1,4] David A. McClellan,[1] Byron J. Adams,[2] Raphael P. Viscidi,[3] James C. Demma,[3] and Keith A. Crandall[1,2]

Department of Integrative Biology, Brigham Young University, Provo, Utah 84602[1]; Department of Microbiology and Molecular Biology, Brigham Young University, Provo, Utah 84602[2]; Department of Pediatrics, Johns Hopkins Hospital, The Johns Hopkins Medical School, Baltimore, Maryland 21287[3]; and Department of Computer Science, Washington University, St. Louis, Missouri 63130[4]

Seventy-two full genomes corresponding to nine mammalian (67 strains) and two avian (5 strains) polyomavirus species were analyzed using maximum likelihood and Bayesian methods of phylogenetic inference. Our fully resolved and well-supported (bootstrap proportions > 90%; posterior probabilities = 1.0) trees separate the bird polyomaviruses (avian polyomavirus and goose hemorrhagic polyomavirus) from the mammalian polyomaviruses, which supports the idea of spitting the genus into two subgenera. Such a split is also consistent with the different viral life strategies of each group. Simian (simian virus 40, simian agent 12 [Sa12], and lymphotropic polyomavirus) and rodent (hamster polyomavirus, mouse polyomavirus, and murine pneumotropic polyomavirus [MPtV]) polyomaviruses did not form monophyletic groups. Using our best hypothesis of polyomavirus evolutionary relationships and established host phylogenies, we performed a cophylogenetic reconciliation analysis of codivergence. Our analyses generated six optimal cophylogenetic scenarios of coevolution, including 12 codivergence events ($P < 0.01$), suggesting that *Polyomaviridae* coevolved with their avian and mammal hosts. As individual lineages, our analyses showed evidence of host switching in four terminal branches leading to MPtV, bovine polyomavirus, Sa12, and BK virus, suggesting a combination of vertical and horizontal transfer in the evolutionary history of the polyomaviruses.

Members of the family *Polyomaviridae* (polyomaviruses) are small, nonenveloped, double-stranded DNA viruses, which are widely distributed among vertebrates. They share a common genome structure consisting of a 4.8- to 5.5-kbp circular double-stranded DNA that encodes five main proteins: two multifunctional regulatory proteins referred to as the large and small T antigens and three structural proteins (VP1, VP2, and VP3), which form the icosahedral viral capsid. At the initiation of this study, at least 16 polyomavirus species were described, but a full-length genomic sequence was available for only 11 (Table 1). Those were nine mammalian and two avian polyomaviruses. Among the mammalian polyomaviruses, there were three full-length genomes available from simian hosts. Lymphotropic polyomavirus (LPV) or African Green monkey polyomavirus was first isolated from a B-lymphoblastoid cell line derived from an African green monkey (43, 75). Serological surveys have revealed that many monkeys and apes, as well as humans, show evidence of infection by viruses antigenically related to LPV (67). However, the natural host of the original isolate and the pathogenicity of LPV are unknown at present. Simian virus 40 (SV40) was identified as a contaminant of monkey kidney cultures used to prepare the first poliovirus vaccines during the late 1950s (54, 65). The natural host of SV40 is the rhesus macaque (56). Simian agent 12 (Sa12, baboon polyomavirus type 1) was first isolated from kidney cells of a vervet monkey (69), but the chacma baboon is con-

sidered the natural host because only this species has shown high titers of Sa12 antibodies (3). We recently sequenced the full-length genome of this virus and included these data in the present study. Subsequent to our effort, the complete genome of a slightly different variant of Sa12 was reported by Cantalupo and colleagues (5). Our sequence and the published genome only differ in the viral noncoding regions. An antigenically distinct polyomavirus, referred to as the baboon polyomavirus type 2, was isolated from cultures of baboon kidney cells (17). A complete genome sequence is not available for this virus, so it will not be included in this study. Several polyomaviruses with available full-length genomic sequences have been obtained from rodents. Hamster polyomavirus (HaPV) was originally described as a virus associated with skin epitheliomas of laboratory colony-bred Syrian hamsters (18). A search for the virus reservoir in weanling hamsters demonstrated virus in the spleen and thymus but, distinct from other mammalian polyomaviruses, not in the kidney (48). Mouse polyomavirus (MPV) was first identified as the etiological agent of a wide range of solid tumors in newborn mice injected with cell extracts of leukemic tissues (22, 59). The virus has been studied extensively as a model agent of cell transformation and virus-host interactions leading to the development of tumors (1). The Kilham strain of mouse polyomavirus or murine pneumotropic polyomavirus (MPtV) is a second murine member of the polyomavirus family (35). MPtV, in contrast to other mammalian polyomaviruses, can cause severe disease. Infection of newborn mice causes interstitial pneumonia with high mortality (19). Unique to this species, the virus replicates in vascular endothelial cells of the lung, liver, and spleen (21). However, in immunocompetent mice, MPtV leads to a persis-

* Corresponding author. Mailing address: 157 Widtsoe Building, Department of Integrative Biology, Brigham Young University, Provo, UT 84602-5181. Phone: (801) 422-3478. Fax: (801) 422-0090. E-mail: mp323@byu.edu.

TABLE 1. Biological characteristics of the polyomaviruses included in this study

Virus name	Abbreviation	Natural host	Species specificity	Tissue specificity	Pathogenicity in normal host
Lymphotropic polyomavirus or African Green monkey polyomavirus	LPV	Human and nonhuman primates	High	B lymphocyte	?Inapparent/mild
Simian virus 40	SV40	Rhesus macaque	High	Kidney	Inapparent/mild
SA12 or Baboon polyomavirus type 1	Sa12	Baboon	High	Kidney	Inapparent/mild
Hamster papovavirus	HaPV	Hamster	High	Kidney/skin	Inapparent/mild or skin epithelioma
Mouse polyomavirus	MPV	Mouse	High	Kidney	Inapparent/mild
Murine pneumotropic virus or Kilham mouse polyomavirus	MPtV	Mouse	High	Lung/kidney	Inapparent/mild or pneumonitis
Bovine polyomavirus	BPV		High	Kidney	Inapparent/mild
JC virus	JCV	Human	High	Kidney	Inapparent/mild
BK virus	BKV	Human	High	Kidney	Inapparent/mild
Avian polyomavirus	APV	Birds (no goose)	Low	Kidney/small intestine	Lethal/multiorgan involvement
Goose hemorrhagic polyomavirus	GHPV	Goose	High	Kidney	Lethal nephritis/enteritis

tent and unapparent infection (19) and the virus localizes mainly to the kidney following primary infection (21), as seen in other mammalian polyomavirus infections. Full-length genomic sequence data are available from one polyomavirus species infecting bovids (49). Bovine polyomavirus is a frequent contaminant of commercial bovine serum (50); it has no known clinical significance for bovids. Two mammalian polyomaviruses are known to infect humans. In 1971, JC virus (JCV) was isolated from the brain of a patient with progressive multifocal leukoencephalopathy (40), and in the same year, BK virus (BKV) was cultivated from the urine of a renal transplant recipient (16). The viruses are ubiquitous, with BKV seroprevalence reaching nearly 100% within the first 5 years of life and JCV seroprevalence approaching 70% by early adulthood (62). The route of transmission is uncertain but most likely either fecal, oral, or respiratory (for a review, see reference 55). Primary infections with JCV and BKV are generally asymptomatic. The viruses persist indefinitely in the infected individual, primarily but perhaps not exclusively in the kidney and are reactivated in times of immunologic impairment. Disease occurs largely in immunocompromised individuals. Progressive multifocal leukoencephalopathy is a rare demyelinating fatal disorder of the central nervous system caused by JCV, which was a frequent complication of human immunodeficiency virus infection prior to the advent of effective antiviral therapy. BKV infection has been associated with hemorrhagic cystitis, ureteral stenosis, nephritis/nephropathy, and less commonly, pneumonitis. Mammalian polyomaviruses for which none or only partial sequence data are available include rabbit polyomavirus (25), rat polyomavirus (72), baboon polyomavirus type 2 (17), cynomolgus polyomavirus (70), and the recently described chimpanzee polyomavirus (32). Those are not included in this study. Avian polyomaviruses (APV) were first identified in psittacine species (2) and latter in a wide range of bird species (33, 61). The prototype APV is the budgerigar fledging disease virus, which is responsible for a fulminating disease in neonate budgerigars (37). A closely related (34) and also lethal (23) polyomavirus is the goose hemorrhagic polyomavirus (GHPV), which causes the hemorrhagic nephritis enteritis of geese. The biology of the avian polyomaviruses

appears to be markedly different from that of the mammalian polyomaviruses. APV exhibits a broad host range compared to the highly specific host range of most mammalian polyomaviruses. Compared to asymptomatic infection, which is characteristic of mammalian polyomaviruses in immunocompetent hosts, the avian polyomaviruses are frequently associated with acute fatal disease. However, because surveys of polyomavirus infection in avian species have not been conducted, the existence of asymptomatic infection cannot be excluded.

Mammalian polyomaviruses adhere to a persistent life strategy (71). They cause unapparent or mild primary infections in young animals, followed by lifelong, nonpathogenic, persistent maintenance of nonintegrated, nondefective, episomal viral DNA (10). They replicate preferentially in the kidneys of a single species or a group of closely related species (4), so they are often described as having cospeciated with their hosts (52, 53, 71). APV, on the contrary, adheres to an acute viral life strategy (71) because it infects a broad array of bird species and is pathogenic (60). GHPV, MPtV, and HaPV do not completely fit these two patterns (Table 1). GHPV, like APV, is the causative agent of a fatal disease in the natural host, but it appears to have a restricted host range like the mammalian polyomaviruses, as it is only known to propagate in goose kidney cells (23). MPtV and HaPV exhibit a unique tissue tropism and the ability to occasionally cause disease. MPtV is unique in its ability to replicate in vascular endothelial cells (20) and cause a fatal lung infection (19). HaPV has the ability to infect hair follicle keratinocytes and cause skin epitheliomas (48), yet each virus exhibits restricted host specificity.

Present assumptions of polyomavirus-host cospeciation are largely the result of the seminal phylogenetic study carried out by Shadan and Villareal (52), although similar observations have been reported previously (58). Their phylogenetic analysis of seven polyomaviruses and their natural hosts (52) showed visual congruence between five mammalian polyomaviruses (HaPV, MPV, BKV, JCV, and SV40) and their host trees, which was taken as an indication of virus-host cospeciation. However, no apparent relationship to the host phylogeny was observed when APV and MPtV were introduced into the analysis, which was attributed to either dislinkage of the viruses

from host evolution or phylogenetic independence of the viruses related to a different tissue specificity (52). Another factor that could also cause the observed phylogenetic incongruence is phylogenetic uncertainty, although this problem was not considered at that point. Subsequent studies and reviews of this topic (53, 71) reaffirm the general view that mammalian polyomaviruses, with the exception of MPtV, cospeciated with their hosts but APV did not and used this assessment to argue about different polyomavirus life strategies. Because of failure to account for phylogenetic uncertainty coupled with lack of a formal statistical hypothesis testing framework for codivergence (for an example, see reference 28), we believe the hypothesis of codivergence between polyomaviruses and their natural vertebrate hosts remains untested.

In this study, we have compared and analyzed all of the 72 complete polyomavirus genomes available (as of October 2005) corresponding to nine mammalian and two avian species using phylogenetic methods that take into account phylogenetic uncertainty. Then, within a statistical framework, we used our best hypothesis of polyomavirus evolutionary relationships and established phylogenies of their hosts to test polyomaviruses-host codivergence using cophylogenetic reconciliation analysis. Our results show how different viral life strategies can be accommodated within a robust hypothesis of polyomavirus-host coevolution.

MATERIALS AND METHODS

Sequence data, alignment, and model selection. Our data set consisted of one new Sa12 complete genome (accession no. DQ435829) and 71 polyomavirus complete genomes from GenBank (Fig. 1). The coding gene sequences were parsed out using CDSparser v1.2 (http://inbio.byu.edu/faculty/dam83/CDSParser /group.asp). The main five genes of the virus genome (VP1, VP2, VP3, large T antigen, and small T antigen) were used for the phylogenetic analyses. Each gene was aligned first at the amino acid level using MUSCLE 3.3 (11), and then the amino acid alignments were converted into nucleotide alignments. Alignment-Helper 1.0 (http://inbio.byu.edu/faculty/dam83/cdm) was used to translate the nucleotide FASTA files to the amino acid level first and then to create nucleotide alignments based on the amino acid alignments produced by MUSCLE 3.3. Gblocks 0.91b (6) was used on each gene nucleotide alignment to assess character uncertainty (i.e., questionable homology statements of aligned ambiguous regions). Ambiguous characters were deleted, rendering a concatenated aligned data set of 5,310 characters. The following Gblocks parameter values were used: p1 (minimum number of sequences for a conserved position), 37; p2 (minimum number of sequences for a flank position), 37; p3 (maximum number of contiguous nonconserved positions), 21; p4 (minimum length of a block), 9; p5 (allowed gap positions), half, per individual gene nucleotide alignment. The best model of DNA substitution based on the Akaike criterion implemented in Modeltest 3.06 (44) was chosen for each resulting Gblock from each gene and all of them concatenated. The following evolutionary models were selected: HKY (26) plus I (invariable sites) plus Γ (gamma distribution) for VP1, TVM (68) plus I plus Γ for VP2 and VP3, GTR (68) plus I plus Γ for large T antigen, small T antigen, and all the concatenated genes.

Phylogenetic analyses. Polyomavirus evolutionary relationships were estimated using maximum likelihood (ML) and Bayesian coupled with Markov chain Monte Carlo (BMCMC) methods of phylogenetic inference. ML searches (13) of the concatenated gene data set were performed in PAUP* v4.0b10 (66) under the GTR plus I plus Γ model with the following model parameters: π (base frequency)$_A$, 0.317; π_C, 0.198; π_G, 0.223; π_T, 0.262; r (substitution rate)$_{CT}$, 1.793; r_{CG}, 3.095; r_{AT}, 1.517; r_{AG}, 1.918; r_{AC}, 3.889; I, 0.092; α (shape parameter of the gamma distribution), 1.394. We conducted ML heuristic searches with 10 random addition replicates and tree bisection and reconnection branch swapping. Uncertainty in the resulting ML relationships was assessed using the nonparametric bootstrap procedure (12) with 100 bootstrap replicates, tree bisection and reconnection branch swapping, one random addition replicate, and starting trees obtained by neighbor joining (using model-corrected distances). BMCMC searches (30) of the unlinked five virus genes were performed in MrBayes v3.0

(47) under the models above. This approach offers several practical advantages over more traditional hill climbing heuristic searches, including simultaneous assessment of both tree and clade support and the ability to accommodate phylogeny and model uncertainty (28). Model parameters were treated as unknown variables with uniform priors and were estimated as part of the analysis. We ran four Markov chains (4.0×10^6 cycles) simultaneously, which were started from random trees and sampled every 1,000th cycle. To check that stationarity had been reached, we monitored the fluctuating value of the likelihood and all the phylogenetic parameters graphically using Tracer v1.2 (46) and repeated each simulation four times starting from different random trees. All sample points prior to reaching stationarity were discarded as "burn in." The posterior probabilities for individual clades obtained from separate analyses were compared for congruence (27, 29, 39) and then combined and summarized on a majority rule consensus tree (29).

Alternative phylogenetic hypotheses were compared using the Shimodaira and Hasegawa (S-H) test (57). Ten thousand replicates were performed for each S-H topology test, resampling the partial likelihoods for each site (RELL model) using PAUP*. All trees were rooted using midpoint rooting.

Cophylogenetic reconciliation analysis. A tanglegram (41) composed of a pruned version of our best phylogenetic hypothesis of polyomavirus relationships, including all of the main polyomavirus lineages and published phylogenetic hypotheses of their hosts, was tested using cophylogenetic reconciliation analysis (Fig. 2). The relationships among the main mammal hosts were depicted from previously published BMCMC and ML trees (38) based on a 16.4-kbp data set. Extensive morphological (for examples, see references 14, 15) and molecular (for examples, see references 24, 42, 45) evidence support the relationships among the four simian polyomavirus hosts, as depicted in Fig. 2 (see the TOL website [http://tolweb.org/tree/phylogeny.html] for further information and references).

Cophylogenetic mapping. Reconciliation analysis of the associations established in Fig. 2 was explored through cophylogeny mapping (31) using TreeMap v.2.02 (7). Accordingly, event costs for codivergence, duplication, loss, and switching were set to 0, 1, 1, and 1, respectively, under the assumption that congruence via codivergence is the null hypothesis. Bounds for the reconciliation analysis were set at the maximum possible: 77 maximum noncodivergence events, a minimum of 0 lineage codivergences, 20 lineage duplications, 10 host switches, 77 lineage losses, and a maximum parasite load of 11. The significance of each reconciliation (jungle) was evaluated in TreeMap via Markov randomization of the virus tree 100 times, bounded by the specific properties of each solution (i.e., number of noncodivergence events, codivergence events, lineage duplications, host switches, and parasite load).

RESULTS

Phylogenetic relationships. ML and BMCMC analyses that account for phylogenetic uncertainty resulted in the same backbone topology (Fig. 1). Evolutionary relationships among different polyomaviruses were strongly supported by high bootstrap (>90%) and posterior probability (pP) (1.0) values. All of the trees showed a major grouping of each of the well-characterized polyomaviruses. BKV and Sa12 formed a clade sister to the JCV and, altogether, to the SV40 clade. MPV and HaPV were sister taxa to a clade of virus infecting monkeys (LPV). More distantly related are the GHPV and APV. Interestingly, the MPtV is clustered with the bovine polyomavirus (BPV), and both are sister taxa to the simian polyomaviruses, excluding the LPV. An alternative rodent monophyletic clade (MPtV, MPV, and HaPV) was significantly rejected by the S-H test ($P < 0.001$) and has a pP value of <0.001. Similarly, a simian polyomavirus monophyletic cluster (BKV, Sa12, JCV, SV40, and LPV) was also rejected (P and p$P < 0.001$). As for the JCV evolutionary relationships, our trees agree with previous phylogenetic hypotheses (63, 64, 73, 74), although our trees showed less resolution among and within types and subtypes and lower clade support. This is not surprising considering that we applied an alignment strategy adequate for assessing deep taxonomic relationships; hence, it is too conservative (i.e., less informative) for resolving JCV relationships.
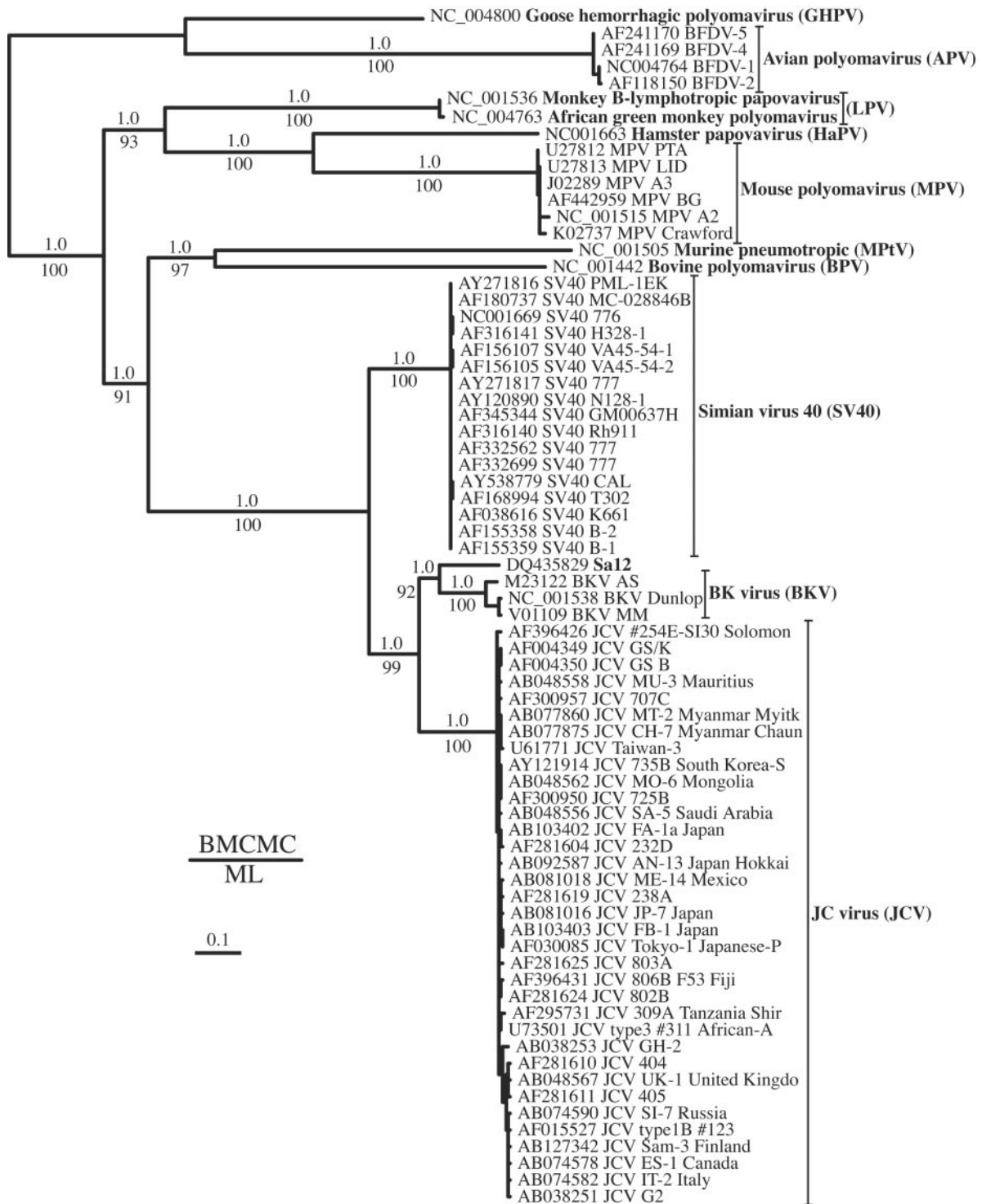
FIG. 1. Midpoint-rooted Bayesian estimation of polyomavirus phylogeny. The same topology was obtained from a maximum likelihood analysis. The branch lengths are drawn proportional to the amount of change along that branch based on the mean branch length distribution (scale shown).

Cophylogenetic mapping and reconciliation analyses. The source phylogenies for hosts and viruses and their associations are depicted in the tanglegram (Fig. 2). Six optimal reconstructions showing a significant degree of congruence ($P < 0.01$)

with their host phylogenies were recovered (Table 2). All of them involved 12 codivergence events and different combinations of host switches and losses per node (Table 2). It is ill advised to discriminate between solutions using the number of

FIG. 2. Tanglegram of polyomaviruses and their hosts with associated taxa connected by dotted lines.
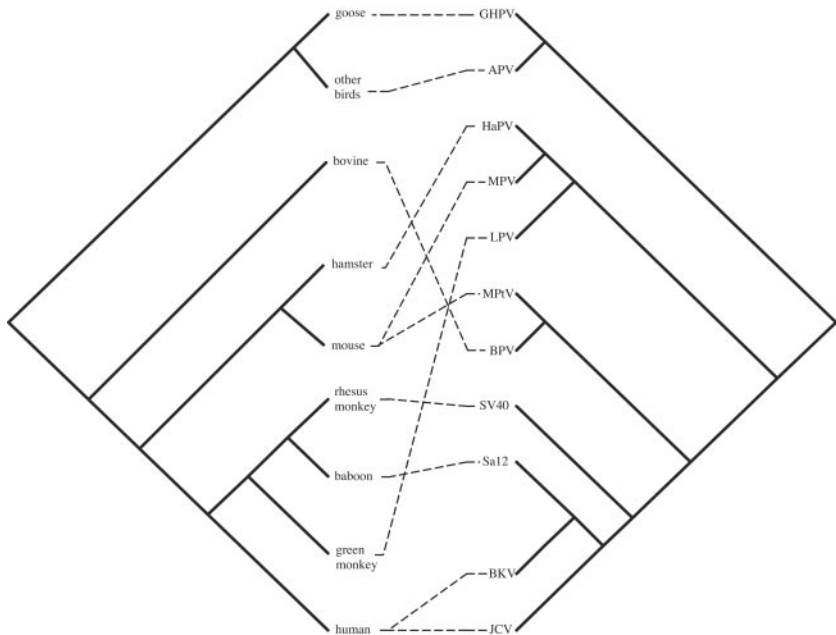
events because noncodivergence events are not necessarily comparable and not all the events are observable (31); hence, a consensus diagram showing the origin of associated lineages across these optimal reconstructions is presented instead (Fig. 3). This diagram summarizes the number of times each lineage arose from each kind of event. Out of 20 lineages, 9 lineages arose from codivergent events and 11 from noncodivergent events, 4 of which were dominated by host switches, 4 by duplications, and 3 by losses.

DISCUSSION

Phylogenetic implications. Our phylogenetic trees based on 72 taxa and 5 genes concatenated (ML) and unlinked (BMCMC) did not agree with previous phylogenetic hypotheses based on the analysis of fewer genes and taxa (23). Our phylogenetic estimate resulted in two reciprocally monophyletic clades separating the polyomaviruses of birds (APV and GHPV) from the mammalian polyomaviruses, which supports the idea of placing these two clades into distinct subgenera (34, 61). This phylogenetic association is also supported by the unique bio-

logical characteristic that separates APV and GHPV from the other polyomaviruses: both are pathogens with a lethal progression (acute life strategy). Rodent and simian viruses do not seem to form monophyletic assemblages, as previously reported (9, 34). Moreover, all of the alternative topologies

TABLE 2. Reconciliation analysis[a]

OR	No. of:					z
	CE	HS	D	L	NCE	
A	12	2	8	6	16	16
B	12	2	8	6	16	16
C	12	4	8	2	14	14
D	12	2	8	6	16	16
E	12	1	8	9	18	18
F	12	0	8	13	21	21

[a] OR, optimal reconstruction ($P < 0.01$); CE, codivergences; HS, host switches; D, duplications; L = losses; NCE, noncodivergence events; z, total event cost (based on assigned event costs).
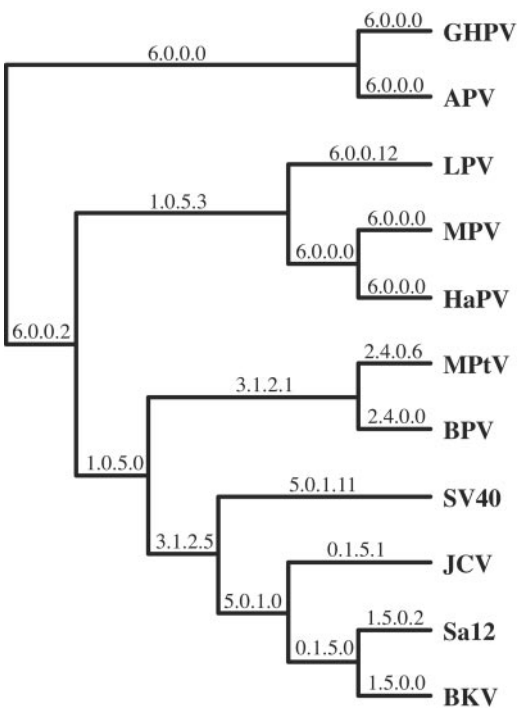

FIG. 3. Consensus diagram showing the origin of associate lineages across six optimal reconstructions resulting from reconciliation analysis. Evolutionary events are separated by periods and presented as follows: codivergences, host switches, duplications, and losses.

grouping these two polyomavirus groups together were significantly worse than our best estimate of phylogeny (Fig. 1). The different evolutionary paths followed by the HaPV, MPV, and MPtV lineages could be driven by their different tissue preferences and/or pathogenicity (Table 1). All simian polyomaviruses but LPV form a robust clade separated by a long branch from their immediate mammal relatives, which fall in an intermediate position in the tree, as indicated by the midpoint rooting. This addresses the fact that, although suspected to be simian, the actual natural host of the LPV is unknown and leaves room for a nonsimian origin of the LPV. If the origin were proven to be murine, host and virus evolutionary paths would be more congruent than reported here.

Codivergence analysis. Previous studies (for example, see reference 52) based on a limited number of taxa and genes did not phylogenetically separate APV and MPtV from their mammalian relatives and concluded that only HaPV, MPV, BKV, JCV, and SV40 cospeciated with their hosts (53, 71). Our more extensive and sophisticated phylogenetic and cophylogenetic analyses showed within a statistical framework that polyomaviruses, as a family, codiverged with their vertebrate hosts (Table 2 and Fig. 3). A basal spilt on the polyomavirus tree separates both avian and mammalian viruses (Fig. 1). Congruence near the base of the reconciled tree (Fig. 2) is an effective indicator that codivergence is the characteristic dynamic, since historically, the signal has not been obliterated by subsequent evolutionary events (31). Such dynamics have been identified in other DNA viruses such as *Herpesvirus* (36). Acute (bird polyomaviruses) and persistent (mammalian polyomavirues) viral life strategies can now be naturally accommodated within this cophylogentic framework.

Mammalian polyomaviruses, as most DNA viruses, adhere to a persistent life strategy (71). Among other biological characteristics (Table 1) they are highly species specific and cospeciate with their hosts. Five lineages in the consensus tree (Fig. 3) leading to MPV, HaPV, MPtV plus BPV, and JCV plus Sa12 plus BKV clades support this view; since they are mostly dominated by codivergence events. However, all the other branches in the tree are dominated by noncodivergence events, and four of those, leading to MPtV, BPV, Sa12, and BKV, are dominated by host switch events. An equal number of switches were observed between MPtV and BPV (2 each way), but four switches were observed from BKV to Sa12 (baboon) and only two were observed from Sa12 to the human BKV and JCV (one each way). Host switching has never been demonstrated in polyomaviruses, so it is assumed that codivergence is the norm. Our analyses suggest that host switching could be more common than expected, at least in recent times. *Hantavirus* of mice, *Spumavirus* of humans (31), and primate lentiviruses (8) indicate that substantial biological boundaries can be overcome under certain circumstances. Nevertheless, our cospeciation analysis does not take into account uncertainty in host-virus associations. Mammalian polyomaviruses were initially isolated from laboratory animals or tissue culture, and little is known about infection in the natural host. For example, BPV was recovered from tissue cultures and is believed to be a contaminant with the calf serum, but no study we are aware of reported its natural host. Some doubt is cast on the bovid origin of BPV by the lack of correlation between detection of viral DNA by PCR and the presence of virus-specific antibod-

ies in serum (51). Similarly, the natural host of the LPV is not known for certain and serological studies have not been able to address this uncertainty. Without such information, it is difficult to judge whether an apparent host-switching is real or the result of isolation from or replication in a transient host. It is thus possible that these apparent cases of host switching might really be examples of misidentification of naturally codiverging hosts. Our phylogenetic analyses may then provide some insight into the origin of these viruses; if, as suggested by our trees, these natural hosts were to be murine, a better fitted coevolutionary pattern would be observed. As for the human-baboon polyomaviruses, all evidence indicates that these are their natural hosts. Hence, if we are to accept a model of codivergence for mammalian polyomaviruses these latter presumptive noncodivergence events will require future mechanistic investigation.

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